THE RELATION OF CERTAIN WEEDS AND GRASSES TO THE DEVELOPMENT OF CEREAL FOOT-ROTTING PATHOGENES IN THE SOIL

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "The Relation of Certain Weeds and Grasses to the Development of Cereal Foot-rotting Pathogenes in the Soil", submitted by Geoffrey Watts Padwick, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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THE RELATION OF CERTAIN WEEDS AND GRASSES TO THE DEVELOPMENT OF CEREAL FOOT-ROTTING PATHOGENES IN THE SOIL

G. W. Padwick

INTRODUCTION

The relation of higher plants to the development of saprophytic soil microorganisms is a subject which has received consideration for a number of years. Since a large amount of the work has been conducted with a view to solving some of the problems of soil fertility, emphasis has been laid largely upon the relation of plant growth to the numbers of microorganisms, and especially to certain groups of bacteria.

The soil is a substratum of very complex nature. It is subject to innumerable combinations of changing physical and chemical conditions, and the microbiological population of the soil is an expression, quantitatively and qualitatively, of these combinations. The total number and the kinds of microorganisms are greatly affected by many known factors, such as available food supply, soil moisture, soil aeration, acidity or alkalinity and

temperature. Every one of these factors is undoubtedly affected by the growth of higher plants in the soil, and in spite of the accumulation of a great amount of data it is still impossible to predict with any very great degree of accuracy the microbial condition of a soil, even when it is possible to determine accurately the physical and chemical conditions. As a result it is realized that a true understanding of soil conditions can only be obtained by a study of the physical and chemical conditions of the soil combined with some sort of measurement of soil microbiological activity. Finally, even when we have got thus far, we find that those who have studied the problem closely are unable to agree upon the best methods of study of activity, one school being of the opinion that biological activity should be measured by the quantity of metabolic products produced in a given time, while the other school holds to the older but more specific method of dilution plate counts.

If relation of higher plants to the general microbial population of the soil is complex, the reactions of certain plant pathogenes to varying conditions in the soil are still more involved. It is necessary to know to what extent any organism to be studied is able to live as a saprophyte in the soil, so that a knowledge of the individual reactions of each microorganism studied to the major changes in soil conditions is required. The present

study was undertaken primarily with the object of ascertaining in what ways the growth of higher plants may modify the soil as a substratum for fungi causing foot-rots of wheat.

Three wheat foot-rotting organisms were selected for the study, namely Ophiobolus graminis Sacc., Helminthosporium sativum P.K.B., and Fusarium graminearum Schwabe. At the commencement of the investigation it was realized that there may be several possible effects of the growth of higher plants on such fungi. Of these, the following are suggested as having an important influence:

1. Higher plants may become infected with wheat foot-rotting organisms in the soil, thus serving as medium well suited for their growth and development to the exclusion of saprophytes which normally, by providing competition, help to prevent any one form from obtaining dominance in the soil. Thus, with the balance upset, the cereal crops following susceptible species may be provided with an abundant source of inoculum.

2. Susceptible plants other than cereals may be an important means by which foot-rotting organisms may persist in the field in the absence of a cereal crop.

3. The pathogenes may spread through the soil more rapidly on or in roots of susceptible plants than in bare soil where the spread is checked by other competitive microorganisms.

4. The roots of plants may secrete compounds capable of stimulating the growth of organisms in the soil. The possibility of the occurrence of the reverse situation, namely, that some plants may secrete substances having an inhibitory effect upon the organisms, must also be considered.

5. Higher plants, either living or dead, may be the chief means of overwintering of the pathogenes in the soil.

WEEDS AND GRASSES AS A MEANS OF SURVIVAL AND SOURCE OF INOCULUM OF WHEAT FOOT-ROTTING ORGANISMS IN THE SOIL

Review of Literature

The relation of plants susceptible to cotton root-rot to the control of the disease (which is of considerable economic importance in the cotton growing districts of the United States) has engaged the attention of plant pathologists in recent years. Taubenhaus and Killough (32) in 1923 stated their belief that clean culture involving destruction of the winter carriers of the causal fungus, Phymatotrichum omnivorum, was of value in helping to control the cotton root-rot disease, while rotations involving only partial freedom from susceptible hosts were not effective. Taubenhaus, Dana and Wolff (31)

in 1929 concluded that it was impossible to control the disease without destroying susceptible perennial weeds. McNamara and Hooton (19) in 1929 found a one-year fallow insufficient, a two-year fallow or a one-year fallow in combination with a rotation system being necessary for control of cotton root-rot.

Henry (11) compared the amount of foot- and root-rot of Marquis wheat grown continuously and in a five-year rotation (wheat, pasture, clover, oats, corn) in Minnesota. The percent of plants diseased on the continuous wheat plots was 58% as compared with 20% on the rotation plots. Sewell and Melchers (26) were compelled to abandon plots in which wheat was grown continuously in an experiment in Kansas owing to the severe infection of foot-rot, while wheat in the rotation division of the same experiment remained free from the disease. Greaney and Bailey (10) in 1924 found that the first crop of wheat after fallow showed considerably less roots infected with root-rot than the succeeding crops. Russel (23) suggested a rotation on new land of breaking, wheat, oats, wheat, summerfallow for controlling take-all in Saskatchewan, and stated that care should be taken to avoid the growth of creeping rooted grasses and volunteer wheat. Sanford (25), as a result of an extended survey in Alberta, found wheat following wheat was heavily infected with root-rot. Infection of wheat following legumes was more severe than expected, and it was

suggested that this might be due to the presence of grass hosts in the legume sod. Russel (24) believed that Bromus inermis (brome grass), Agropyron tenerum (western rye grass), and A. repens (quack grass) all serve to increase the amount of take-all in crops of wheat following them in the rotation.

All three organisms to be studied are able to carry on a saprophytic existence in the soil, but the extent to which they do so under natural conditions is not easily determined.

Kirby (15) found that soil infested with Ophiobolus graminis, when screened and kept indoors for eight months, lost its ability to cause take-all of wheat, while bits of straw containing perithecia were able to cause severe infection when similarly treated. Davis (4) and Russel (22), however, have found this fungus to persist in bare soil for considerable periods.

Davies (3) added Helminthosporium sativum and Fusarium graminearum in the form of mycelium or spores to unsterilized soil in boxes. The boxes were placed in the field and subjected to various treatments. H. sativum was recovered from bare fallow and after wheat, and F. graminearum was recovered from soil kept bare as well as from soil which grew wheat or oats. By one isolation method the soil yielded H. sativum after every treatment, regardless of whether the organism had been added to the soil or not at the commencement of the experiment.

Henry (12) found strong inhibition of H. sativum brought about by the introduction of small quantities of unsterilized black loam soil into pots of sterilized soil to which H. sativum inoculum had been added, and suggested that under natural conditions saprophytic microorganisms probably play a large part in maintaining at a low level the abundance of foot-rotting organisms in the soil. The same writer (13) found that spores of H. sativum occur rarely if ever in field soils, but points out that the fungus may be able to survive in the soil in the form of mycelium.

Results of a number of investigations have given a fairly extended knowledge of the host range of the organisms concerned. Early reports of the occurrence of perithecia of O. graminis on grasses were made by Saccardo, according to Kirby (15), in 1875, Waters (34), and Brittlebank (1). Kirby (15) in 1921 found A. repens commonly affected with take-all in New York state under natural conditions; in addition perithecia were formed on all six species of Agropyron studied, namely A. caninum, A. cristatum, A. intermedium, A. repens, A. Smithii and A. tenerum. In a later paper (16) the list of Agropyron species was extended to include A. desertorum, A. obtusiusculum, A. Richardsonii, A. scabrum and A. spicatum. Perithecia were also produced on Hordeum jubatum (wild barley). Rosen and Elliot (21) in 1925 found several

grasses attacked by Ophiobolus cariceti in Arkansas, and in one instance found Chaetochloa geniculata (perennial foxtail) attacked on land which had never grown wheat. Padwick and Henry (20) called attention to the importance of ascertaining the relative degree of infection caused by foot-rotting organisms in various susceptible grasses in order to obtain a comprehensive knowledge of their importance in the foot-rot problem of Alberta. Bromus inermis, B. ciliatus and all the species of Agropyron studied were severely damaged by O. graminis on both sterilized and unsterilized soil (Plate I). Infection of Agropyron repens with O. graminis was found to be severe and general throughout the take-all areas of Alberta and was commonly associated with unusually heavy infection of wheat with take-all when growing as a weed in the crop (Plate II). In addition this weed was commonly infected with O. graminis in summerfallow fields. Agropyron tenerum (western rye grass) was also severely damaged by O. graminis under natural conditions (Plate III).

Dreschler (5) found that H. sativum commonly caused a leaf spot disease of A. repens in the mid-western United States. Stakman (27) obtained infection of leaves of Agropyron Smithii, A. repens and Hordeum jubatum with H. sativum. Christensen (2) isolated a species of Helminthosporium which he believed to be H. sativum from above ground parts of Agropyron caninum, A. desertorum, A. repens, A. Smithii and A. tenerum, as well as other

PLATE I

Reaction to Ophiobolus graminis of some grasses of economic importance. Check plants at left and plants from infested soil at right. Left to right: Agropyron tenerum, A. repens, A. cristatum, Bromus inermis, Hordeum jubatum and Phleum pratense.

PLATE II

Figure 1

Figure 2

Figure 3

Figure 4

PLATE II

Figure 1. A take-all patch in quack grass. The stake in the foreground shows the height of the diseased plants, and that in the background shows the height of healthy plants.

Figure 2. At left, rhizomes of plants from a diseased area; at right, rhizomes of healthy plants.

Figure 3. At left, plants from a diseased area; at right, healthy plants from just outside the diseased area.

Figure 4. A quack grass patch in a field of wheat. Wheat in the quack grass patch is shown at the left, and outside it at the right. About 30% of the wheat plants were killed by take-all where quack grass was present, but only about 5% elsewhere. The stunting of the wheat was due partly to choking out by the weed.

PLATE III

Figure 1

Figure 2

Figure 3

PLATE III

Figure 1. A healthy field of western rye grass.

Figure 2. Killing of western rye grass by take-all. Right foreground, a western rye grass plot, showing dead plants and a number of encroaching weeds; left foreground, timothy; right background, alfalfa; left background, brome.

Figure 3. A closer view of a badly diseased western rye grass plot.

PLATE IV

1

2

3

4

5

6

PLATE IV

Reaction of species of Agropyron to Fusarium graminearum and Helminthosporium sativum on sterilized soil. Rows 1, 2 and 3, from left to right, Agropyron cristatum, A. sibiricum, A. obtusiusculum, A. Smithii, A. desertorum, A. caninum. Rows 4, 5 and 6, from left to right, Agropyron elongatum, A. Richardsonii, A. Griffithsii, A. tenerum, A. dasystachyum, A. repens. Rows 1 and 4, check (no organism added); rows 2 and 5, Helminthosporium sativum; rows 3 and 6, Fusarium graminearum.

grasses, under natural conditions. Seven species of Agropyron all showed infection of leaves to a more or less marked degree when inoculated with H. sativum in the greenhouse. Little attention was given to the amount of root infection. Padwick and Henry (20) found the root systems of all the twelve species of Agropyron studied, and also Bromus inermis and B. ciliatus to be infected with H. sativum (Plate IV). This fungus was also isolated from underground parts of Agropyron repens, A. tenerum, A. Richardsonii, and Bromus inermis growing in the field.

Studies on the effects of Fusarium graminearum on grasses (20) showed a general tendency for the fungus to attack the seeds and destroy them before emergence. Seedlings of species of Agropyron which emerged from the soil seemed to suffer little damage from the fungus, and the organism was rarely reisolated (Plate IV). It was, however, frequently yielded by Bromus inermis and B. ciliatus.

Experimental Methods and Results

Five species of higher plants of economic importance in Alberta were selected in order to determine their effect on the survival of O. graminis, H. sativum and F. graminearum in the soil and their role in increasing the amount of inoculum for infecting wheat. Four of the plants studied were graminaceous species, namely Agropyron tenerum

(western rye grass) and A. cristatum (crested wheat grass), which are cultivated as forage crops in western Canada; A. repens (quack grass), which is a common weed in central Alberta and Bromus inermis (brome grass), which is grown considerably for forage purposes. All these grasses are perennials. The fifth species was a dicotyledonous plant, Neslia paniculata (ball mustard), an annual weed.

The method adopted was to apply inoculum of the various organisms to pots of soil, together with seed of the species to be studied, and after a period of growth varying from five to eight weeks to seed the pots to wheat, which served as an indicator of the survival of the fungus in the soil and of its ability to reinfect wheat in planted soil as compared with controls in unplanted soil. Some experiments were run concurrently, using the same controls; others were run separately and separate checks had to be used. In certain instances, owing to unsuitable temperatures obtained in the greenhouse while the experiments were being conducted, some series, together with their checks, had to be repeated in their entirety.

The inoculum was prepared by growing the organisms on black loam soil in Erlenmeyer flasks, 50 grams per flask, to which was added 28 cc. of tap water. The flasks were plugged with cotton batting and sterilized in the autoclave before adding the fungus. The fungus was allowed to grow in the flask for 17 days at room temperature. Six-inch pots

were half filled with black loam sod and half of the pots were then sterilized in the autoclave for four hours. Each pot then had added to it as inoculum the entire contents of an Erlenmeyer flask. Seeds of the plant species to be studied were then placed in the pot and covered with soil. Ten replicates of each treatment were seeded, so that every species under investigation was grown in ten pots with each of the three fungi on sterilized soil, and ten pots on unsterilized soil. Similar pots were filled and had fungi added in a similar manner, but were not seeded, and these were used for comparison with the seeded pots. All the pots were then placed in the greenhouse, and the plants were allowed to grow for five to eight weeks. The top growth of the plants was then cut off and each pot was seeded with 25 seeds of Marquis wheat, which had previously been treated with hot water in order to kill as much as possible of any pathogenic fungi which might be present in or on the seeds. After three weeks the wheat seedlings were harvested and measured (from the base of the subcoronal internode to the tip of the longest leaf) and were awarded infection ratings which were later averaged for each pot and the series of pots and converted into degree of infection in percent. The results of these experiments are found in Appendix I to V, and are further summarized in Table 1.

Table 1

Infection of wheat with Ophiobolus graminis, Helminthosporium sativum and Fusarium graminearum following certain weeds and grasses.

Cropping	Soil	Presence of grass or weed	Agropyron tenerum		Agropyron cristatum		Agropyron repens		Bromus inermis		Nealia paniculata	
			Degree of infection of wheat plants in percent	Probability (1)	Degree of infection of wheat plants in percent	Probability (1)	Degree of infection of wheat plants in percent	Probability (1)	Degree of infection of wheat plants in percent	Probability (1)	Degree of infection of wheat plants in percent	Probability (1)
[illegible]	Unsterilized	Absent	0		0		0		0		0	
[illegible] graminis	Unsterilized	Present	11.3	++	1.8	+	5.2	++	9.9	++	0	---
	Sterilized	Absent	0.4		0.4		0.4		0.4		0	
	Sterilized	Present	13.0	++	5.8	++	6.7	++	15.9	++	0	---
	Unsterilized	Absent	9.4		9.4		9.4		5.6		5.0	
Helminthosporium sativum	Unsterilized	Present	14.5	0	15.3	0	15.4	+	10.1	++	4.9	0
sativum	Sterilized	Absent	3.3		3.3		3.3		31.8		30.7	
	Sterilized	Present	14.7	++	9.8	++	9.6	++	51.0	++	30.3	0
	Unsterilized	Absent	17.5		17.5		17.5		3.6		7.2	
Fusarium	Unsterilized	Present	23.0	0	22.2	0	20.0	0	27.1	++	7.8	0
graminearum	Sterilized	Absent	30.6		30.6		30.6		44.9		31.4	
	Sterilized	Present	26.1	0	20.2	0	32.5	0	41.1	0	21.4	0

(1) Probability refers to the odds according to the method of Student. Odds below 30:1 are considered to mean that the difference is not significant (0); odds of 30:1 to 200:1 mean a significant difference (+); and above 200:1, very significant (++).

In each table (Appendix I to V) the average height of the wheat plants grown after other plants is directly comparable with the height of the wheat plants when grown in bare soil (column 4). The probability of the significance of the difference has been calculated by Student's method and is given for each pair (column 5). The average degree of infection in percent is found in column 6, and the probability that the difference is significant is found in column 7.

Considering the three organisms separately, it is seen that in the case of Ophiobolus graminis there was in every case a very marked and significant increase in the degree of infection of wheat following grasses, on both sterilized and unsterilized soil. In fact, in the case of unsterilized soil, there was no trace of infection in the absence of weeds. It is seen that in most cases there was only a slight reduction in height of wheat plants. However, had a longer period elapsed before planting the wheat it is quite conceivable that differences in infection of wheat plants, and consequently in height of plants, would have been even greater. It is seen that Neslia paniculata effected no significant difference in the infection of wheat with take-all.

With Helminthosporium sativum the most striking results were obtained on sterilized soil. After all the grasses, with the exception perhaps of Bromus inermis, a very marked increase of infection was accompanied by a marked decrease in height of the plants. On unsterilized soil the grasses effected only a small increase in infection, and excepting Bromus inermis, no decrease in height of plants. There was no increase in infection with Neslia paniculata.

There was no significant increase of Fusarium graminearum foot-rot as a result of the growth of species of Agropyron, nor was there any increase caused by growth of Neslia paniculata. Wheat after Bromus inermis in

unsterilized soil, however, showed a striking increase of Fusarium foot-rot and a marked decrease in height of the plants. There was no increase of infection in sterilized soil, but in view of the fact that on bare sterilized soil the infection reached 44.9%, a great increase would perhaps not be expected.

The results in general show a close agreement with what was to be expected from results of experiments previously reported (20). In those experiments it was found that all the species of Agropyron studied were severely damaged by O. graminis, as also was Bromus inermis. The present results show that on bare unsterilized soil the organism under the conditions existing seemed to have disappeared, while on soil planted to A. tenerum, A. cristatum, A. repens and B. inermis considerable infection of the wheat occurred. In all cases a marked increase occurred in the degree of infection of wheat with O. graminis on unsterilized soil after susceptible grasses, indicating a tendency of the organism to accumulate in soil planted to these grasses.

In the previous experiment all the four grasses studied were found susceptible to H. sativum, and all have served in the present experiments to increase the amount of inoculum of this organism on sterilized soil. The amounts of increase on unsterilized soil were not very significant. It was previously found that none of the four grasses appeared to be damaged by H. sativum on

unsterilized soil to the same extent as they were by O. graminis, and for this reason it was only to be expected that the grasses would play a somewhat smaller role in carrying over the organism and serving as a source of inoculum for the succeeding wheat. B. inermis was the only grass of the four studied which was infected and damaged by F. graminearum on unsterilized soil, and it was the only grass which served to increase the amount of inoculum of this organism in unsterilized soil. In no instance did Neslia paniculata serve to aid in the survival or increase of any wheat foot-rotting pathogenes in the soil.

These results support the view expressed earlier in this thesis that the mere fact that a plant is susceptible to attack by wheat foot-rotting pathogenes under the unusual conditions of experimental inoculation, especially on sterilized soil, is not adequate in giving an indication of the role which it may play in the foot-rot problem of wheat. It is necessary to obtain an indication of the relative amount of damage done to these susceptible plants, on unsterilized as well as sterilized soil, before a reliable estimation of their importance in the problem may be made.

THE EFFECT OF GRASSES ON THE HORIZONTAL SPREADING OF CEREAL FOOT-ROTTING ORGANISMS

Review of Literature

The spread of microorganisms in the soil is a subject which for many years has aroused the interest not only of workers in the field of microbiology, but also those interested in the wider fields of general biology and agriculture. The typical "fairy rings" of lawns and pastures have aroused curious interest for centuries, but it is only comparatively recent knowledge which has shed light on the cause and nature of the phenomenon. These rings are now known to be due to the growth of fungi of the class Basidiomycetes.

Microorganisms vary greatly in their ability to spread from one point to another in the soil. What little evidence we have concerning bacteria suggests that possibly their rate of spread in the soil is small. Frazier and Fred (8) attempted to determine the rate of spread of the root nodule-forming bacteria of legumes in soil and found that in flat iron boxes in which movement of water was reduced to a minimum the organisms spread only 0.1 to 0.2 inches a day. Sterilized limed yellow sand was used in the boxes. In addition, in a field

experiment in which soy beans were planted three feet apart in each direction, alternate plants were inoculated with B. radicicola. With one exception, after about three months from the date of seeding, the uninoculated plants showed no sign of nodules, while inoculated plants were thickly studded with nodules.

More definite demonstrations of ability to spread in soil is afforded by fungi. Mention has already been made of the fungi causing "fairy rings", which tend to increase in diameter as the organisms progress in the soil until they have reached many feet in diameter. One of the most remarkable fungi reported in this connection is the cotton root-rot fungus, Phymatotrichum omnivorum, which also attacks alfalfa and many other dicotyledonous plants. In fields of alfalfa in Arizona King (14) in 1923 found this organism spreading rapidly in almost perfect circles. In cotton fields the organism spread in all directions from single infected plants at the astounding rate of 4½ meters in 50 days. McNamara and Hooton (19) in 1929 found that the most active killing of plants occurred always on the outer edge of the circle, the plants in the ring of the previous year's growth not being killed until late in the season and in some cases remaining unharmed when the weather was unfavourable for the growth of the fungus. It was suggested that the fungus either used up all the available food as it progressed or else left a toxin in the soil retarding its growth.

The difficulty of isolating Ophiobolus graminis from the soil and from infected plants, its weak saprophytic growth, and the inconspicuous nature of the symptoms of the disease it causes when in the early stage, have made a knowledge of the distribution and progress of the organism in the soil under field conditions difficult to obtain. Russel (23) in 1928 was unable to demonstrate the spread of take-all from inoculated seedlings in the center of eight-inch crocks to seedlings sown closely around them, the neighbouring plants reaching maturity without showing symptoms. Fellows (6) has found the organism present to a depth of at least ten inches in infested soils in Kansas, but it is possible that the organism may have been carried to this depth by deep ploughing. It seems to be the popular belief that the circular patches of diseased wheat plants in fields are the result of the radial growth of the organism from a center of infection. Similar circular spots in A. repens have been observed (20) to be very marked in a field which had remained uncultivated for several years and was supporting a thick stand of this weed (Plate II, Figure 1).

Experimental Methods and Results

Improved technic in isolating Ophiobolus graminis from the soil has made possible a study of the conditions under which this organism survives and spreads. Extensive

experiments were outlined to determine whether this organism, and also Fusarium graminearum, are able to spread progressively through unsterilized soil, both when bare and when planted to various grasses and to wheat.

Owing to the extensiveness of the experiment and the large amount of isolation work involved, the experiments were conducted with the two organisms at different times, first with F. graminearum (in the summer of 1932) and then with O. graminis (in the winter of 1932-'33). There were, however, no essential differences in the methods adopted in each case. On June 28th fifteen flat wooden boxes, size 25 x 16 x 3½ inches, were filled to a depth of 2½ inches with unsterilized black loam soil, obtained from land kept bare for five years. Three boxes were seeded with wheat, three with Agropyron tenerum, and three with A. repens. About 200 seeds were sown in each box. The remaining six boxes were not seeded. All were placed in the greenhouse at a soil temperature of about 20°C. and kept watered. On July 29th, after the plants had become well established, a narrow trench was dug to the full depth of the soil, two inches from one end and across the full width of each box. In this was placed inoculum of F. graminearum, prepared by growing the fungus for 17 days in Erlenmeyer flasks each containing 50 grams of moist sterilized soil plus 10% of cornmeal. Two flasks were used for each box. Three of the unplanted boxes were treated in a precisely similar manner, while the remaining

three had placed in the trenches a similar quantity of sterilized soil and cornmeal which, however, had no fungus growing on it. Thus it was possible to compare the spread of the organism in the presence of the two grasses, in the presence of wheat, and in unplanted soil. The purpose of the uninoculated boxes was to check against the natural occurrence of the fungus in the soil, which would at once have rendered the results valueless. On September 7th, after removing the top growth, Marquis wheat which had been treated with hot water to reduce the seed-borne foot-rotting fungi to a minimum, was seeded in rows across the flat along the original trench, and in rows parallel to it and two inches apart. Twelve rows were seeded in all. Twenty seeds were placed in each row, a small hole being made for each seed with a wooden meat skewer, a new meat skewer being used for each row. On September 27th the wheat plants from one box under each treatment were dug up and measured, the degree of infection of each plant was recorded, and underground parts of plants in all rows showing any trace of foot-rot were removed. These underground parts were surface sterilized by dipping for 1½ minutes in mercuric chloride (1 gm. in a liter) and washing in 75% alcohol. The second group of boxes was harvested and treated similarly on October 1st, and the third replicate on October 6th. The average length and the average degree of infection in percent of each row of wheat plants, together with the results of the isolations are shown in Appendix VII.

Table 2

Results of Experiments on the Spread of *Ophiobolus graminis* in Planted and Unplanted Soils.

Soil treatment	Distance in inches from original place of application of inoculum	Average height of wheat plants in cms.	Average degree of infection of wheat plants in %	Results of re-isolation trials of *O.graminis* Maximum distance of spread
Soil left bare. Cornmeal soil with no organism was placed at one end of the box. Included to check against natural occurrence of *O. graminis* in the soil.	0	26.0	0	-
	2	27.3	0	-
	4	23.2	0	-
	6	23.4	0	-
	8	23.8	0	-
	10	24.3	0	-
	12	21.8	0	-
	14	23.0	0	-
Soil left bare. *O. graminis* placed at one end of the box.	0	23.5	14.4	+
	2	24.1	1.1	+
	4	24.0	0	-
	6	23.8	0	-
	8	25.4	0	-
	10	23.2	0	-
	12	22.1	0	-
	14	23.7	0	-
Soil seeded to wheat. *O. graminis* placed at one end of the box.	0	28.8	14.1	+
	2	24.0	29.3	+
	4	20.0	29.4	+
	6	19.3	15.4	+
	8	22.0	7.7	+
	10	21.1	0.6	-
	12	21.5	0	-
	14	21.6	0	-

Table 5

Results of Experiments on the control of <u>Pratylenchus</u> <u>penetrans</u> in Planted and Unplanted Soils.

Table 2 (Continued)

Soil treatment	Distance in inches from original place of application of inoculum	Average height of wheat plants in cms.	Average degree of infection of wheat plants in %	Results of re-isolation trials of *O. graminis* Maximum distance of spread
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of the box.	0	25.6	9.7	+
	2	21.6	24.7	+
	4	21.2	10.7	+
	6	18.8	8.8	+
	8	19.1	7.2	+
	10	17.9	0.7	+
	12	18.9	0.6	+
	14	18.8	0	-
Soil seeded to *A. repens*. *O. graminis* placed at one end of the box.	0	26.3	8.6	+
	2	26.8	18.9	+
	4	21.2	13.2	+
	6	20.8	1.0	-
	8	20.2	0	-
	10	21.0	0	-
	12	20.6	0	-
	14	17.8	0	-

Table 3

Results of Experiments on the Spread of Fusarium graminearum in Planted and Unplanted Soils.

Soil treatment	Distance in inches from original place of application of inoculum	Average height of wheat plants in cms.	Average degree of infection of wheat plants in %	Results of re-isolation trials of F. graminearum
Soil left bare. Cornmeal soil with no organism placed at one end of box. Included to check against natural occurrence of F. graminearum in the soil.	0	32.0	1.1	-
	2	26.2	3.6	-
	4	28.3	1.1	-
	6	25.5	1.0	-
	8	24.2	4.8	-
	10	24.7	4.4	-
	12	26.6	2.3	-
	14	26.9	3.4	-
	16	27.2	1.1	-
	18	26.6	2.5	-
Soil left bare. F. graminearum placed at one end of box.	0	27.5	11.9	+
	2	28.5	3.8	-
	4	27.9	12.6	-
	6	27.6	2.4	-
	8	27.1	1.3	+
	10	27.4	5.0	-
	12	22.6	1.0	-
	14	27.2	6.3	+
	16	22.6	3.2	+
	18	27.1	1.1	-
Soil seeded to wheat. F. graminearum placed at one end of box.	0	27.9	24.8	+
	2	27.6	16.9	+
	4	25.1	24.1	-
	6	23.0	17.4	-
	8	22.2	19.0	-
	10	20.8	22.9	-
	12	19.0	19.4	-
	14	22.3	12.1	+
	16	22.2	16.6	-
	18	21.3	16.7	-

Table 2

Results of Experiments on the Effect of Applying Fertilizer in Placed and Broadcast Bands.

Soil Treatment	Pounds of Nitrogen per Acre Applied at Seeding	Average Height of Plants in Inches.	Average Yield of Tops in Pounds per Acre.	Probable Error of Average Yield.
Soil left bare.	0	32.1	1.1	-
Covered soil with no crystals	0	32.6	0.5	-
Placed at one side of box.	5	35.2	1.1	-
Included to check against several centrals	10	36.0	1.0	-
	10	34.1	0.6	-
	10	35.7	0.4	-
	15	40.1	0.8	-
	15	39.4	0.6	-
	15	39.4	1.1	-
	15	39.4	0.6	-
Soil left bare.	0	39.3	21.3	-
Placed at one end of box.	5	39.7	1.8	-
	5	39.4	1.3	-
	5	37.4	0.4	-
	5	36.1	0.4	-
	10	36.9	0.0	-
	15	38.3	0.0	-
	14	37.1	0.0	-
	16	37.0	0.0	-
	15	36.4	0.1	-
Soil sealed to	0	33.0	24.3	-
Placed at one side of box.	5	34.3	26.4	+
	5	33.1	24.1	-
	5	33.0	21.4	-
	5	33.3	23.0	-
	10	30.0	24.3	-
	15	29.3	23.4	-
	14	31.3	12.1	-
	15	32.3	12.6	-
	15	31.5	19.7	-

Table 3 (Continued)

Soil treatment	Distance in inches from original place of application of inoculum	Average height of wheat plants in cms.	Average degree of infection of wheat plants in %	Results of re-isolation trials of *F. graminearum*
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of box.	0	26.7	27.9	+
	2	26.5	21.2	+
	4	25.8	21.4	+
	6	23.4	26.1	-
	8	22.6	16.7	-
	10	24.1	12.9	+
	12	24.1	11.1	+
	14	24.0	10.9	-
	16	22.5	17.2	-
	18	22.9	18.5	-
Soil seeded to *A. repens*. *F. graminearum* placed at one end of box.	0	26.6	26.9	+
	2	27.2	27.5	+
	4	25.2	23.2	-
	6	24.9	30.8	+
	8	23.1	32.4	-
	10	24.4	27.4	-
	12	24.0	32.1	-
	14	23.5	23.9	-
	16	24.1	31.0	-
	18	22.9	17.2	+

A few slight modifications were made for O. graminis. As stated previously, the experiment was conducted during the winter, being commenced about November 1st. Inoculum of O. graminis (strain 108, obtained from A. repens) was added November 29th. In order to minimize washing of inoculum over the soil in the boxes, the boxes were tilted slightly, so that if the organism were carried at all by the water it would be in the opposite direction to that in which the rate of spread was to be determined. Only ten rows of wheat were seeded, on January 20th, fifty-two days after adding the inoculum to the end of the box. All three replicates were harvested on February 14th. In sterilizing for re-isolation, silver nitrate was used instead of mercuric chloride. Small portions of roots were dipped in silver nitrate solution (1 gram in 100 cc. of water) for one minute, and the silver nitrate was then precipitated with concentrated sodium chloride solution, after which they were plated in petri dishes. Results are shown in Appendix VI.

The averages of lengths of plants and degree of infection in percent for the three replicates of each treatment are further summarized in tables 2 and 3. The distances of spread of O. graminis in each box under the various treatments are shown in table 4.

Table 4

Distances to which *O. graminis* Spread in Two Months in Bare Soil and in Soil Seeded to *A. tenerum*, *A. repens*, and wheat.

Soil treatment	Distance in inches (to nearest 2 inches) to which *O. graminis* spread in the soil		
	Series 1	Series 2	Series 3
Bare soil	0	2	0
Wheat	6	8	8
A. tenerum	4	12	8
A. repens	4	4	4

The results with *O. graminis* are striking and significant. Not only are there very great differences in the distances the organism spread under different treatments, but also there is in most cases quite close agreement between the replicates of each series. Appendix VI shows that the organism spread in only one of the three boxes of unplanted soil, and there it only spread about two inches to cause a small amount of infection of wheat. In boxes growing wheat it spread six inches in one box and eight inches in the other two; under *A. tenerum* it spread four, twelve and eight inches; and in all boxes of *A. repens* it spread four inches. The organism was in no instance isolated from wheat plants from the uninoculated flats.

Table 4

Distances to water *A. gayanthi* burrows in two sorts of soil from west of Dell Ranch: *A. terricola*, *A. tenuis* and basalt.

Soil treatment	Distance to burrow (in inches) to nearest *A. gayanthi* burrow in each soil		
	Series A	Series B	Series C
Bare soil	0	0	0
Tuber	0	0	0
A. terricola	0	12	0
A. tenuis	0	0	0

The results with *A. gayanthi* are striking and significant. Not only are there very great differences in the distances the occupied burrows are from the three treatments, but also there is in most cases quite close agreement between the replications in each series. Opposite to those that the optimum spread is only one of the three doses of reclaimed soil, but there it is given about the inches to which moisture of tubers of basalt. To doses growing water it spread six inches in one and eight inches in the other two; under *A. terricola* it spread four. Twelve and eight inches; not in all cases of *A. tenuis* it spread that inches. The response was to identify isolated from which plants that contaminated there.

PLATE V

Figure 1

Figure 2

Spread of Ophiobolus graminis in unsterilized soil. Left to right, row seeded along infestation trench, 2 inches away, 4 inches away, etc. Figure 1, wheat seeded on bare soil; Figure 2, wheat seeded after wheat.

An interesting point is demonstrated in the degree of infection columns of Appendix VI and table 2. It is seen that in almost all planted boxes there was less infection along the rows where the inoculum was added than there was two or four inches away (Plate V). This may have been due to one or all of several causes. The wheat plants may have been stimulated by the cornmeal added to the soil with the inoculum, and thus have grown so rapidly as to overcome to some extent the effects of the organism; the cornmeal may have stimulated growth of saprophytic organisms detrimental to O. graminis; cutting out the trench destroyed some of the grass and wheat plants originally in the soil, and may thus have reduced the amount of preferential medium for growth of O. graminis; or there may have been a tendency, as suggested by McNamara and Hooton (19) in Phymatotrichum omnivorum, for the greatest damage to be caused in the neighbourhood of the most recent advances of the organism. The latter possibility is one well worthy of further investigation. In any case, it is seen that there is a marked tendency for O. graminis to spread in soil planted to wheat or susceptible grasses, whereas on unplanted soil there appears to be little or no tendency to spread.

Results with F. graminearum were irregular and less conclusive. Unfortunately several important points were overlooked in conducting the experiment. While considerable care was taken in watering the boxes, they

were not sloped and some washing of soil no doubt occurred. The effects of this were accentuated by the fact that the inoculum contained spores. That these spores were carried for considerable distances is evidenced by the fact that single infected plants were found isolated from other infected plants, by as much as fourteen inches in one instance. The F. graminearum causing this infection evidently came from the original inoculum, since in no instance was the organism isolated from uninoculated soil. Another mistake was that of allowing the wheat plants for isolation to grow for so long a period before removing them from the soil to isolate F. graminearum. Especially in the last two series harvested there was so heavy an infection with Helminthosporium sativum (indicated by H in the isolation column) occurring naturally in the soil that the degrees of infection recorded in the columns of Appendix VII are rendered almost meaningless. The only value of these columns is in demonstrating the increase in damage caused by H. sativum on naturally infested soil following wheat, A. tenerum and A. repens. It should be mentioned here that a similar experiment to those described above was first conducted with H. sativum added to the soil, but the natural occurrence of the organism in the soil rendered the results valueless. It can only be said that there is no definite evidence that the spread of F. graminearum in unsterilized soil is affected by the presence of susceptible plants in the soil.

EFFECTS OF GRASSES ON OVERWINTERING OF THE FOOT-ROTTING FUNGI

Review of Literature

Taubenhaus and Killough (32) pointed out that in the case of cotton root-rot in Texas bare fallow would only be efficient in controlling the disease provided susceptible weeds and plants were kept under control in winter as well as summer. It must be remembered, however, that this organism is capable of infecting healthy plants during the winter weather in Texas, so that overwintering becomes a minor part of the problem in that climate, and the problem resolves itself into a question of the survival of the organism in the soil comparable to the summer survival, but somewhat less intensified owing to the reduced rate of growth. It is interesting to compare the problem of the survival of wheat foot-rotting organisms in the severe climate of Alberta with that of Phymatotrichum omnivorum in Texas.

Davis (4) found Ophiobolus graminis able to overwinter at Madison, Wisconsin, in both mycelial and ascospore stages, but under much less severe conditions than those occurring in Alberta. Foster (7) found that mycelia of Helminthosporium sativum and Fusarium graminearum

survived the severe winter of 1928-29 on barley seeds buried in the soil and on wheat stubble, and that spores of H. sativum and F. graminearum were viable in the spring after overwintering in the open. In the winter of 1931-32, Davies (3) isolated Ophiobolus graminis from naturally infected stubble in the field at various periods throughout the winter. The fungus also survived in mycelial form when grown saprophytically in flasks of sterilized soil and then placed outside for the entire winter.

Presumably, owing to the low temperatures maintained during the winter in Alberta, all organisms must be in a dormant state. Whether susceptible plants are present or not there will necessarily be no growth of the organisms. Furthermore, susceptible perennial host plants cannot be destroyed during the winter. The results of Davies and Foster indicate that the organisms may survive in the soil without the presence of such infected dead material, though it is possible that they may be more susceptible to freezing injury in some substrata than in others. In spring, resumption of growth would probably be more certain if a favourable medium were present. Perennial plants would probably be able to get an early start in the spring and thus the disease might start at the place where it left off in the fall. This seems in the light of present knowledge to be a minor problem, and experiments were therefore not conducted.

THE EFFECTS OF PLANT MATERIALS ON THE GROWTH OF *OPHIOBOLUS GRAMINIS*

Review of Literature

The conditions of the soil for growth of micro-organisms are constantly undergoing change as a result of many factors, one of which is the growth of higher plants. That higher plants can and do exercise a marked influence on the abundance and type of the soil microflora has been amply demonstrated.

Wilson and Lyon (35) showed that the growth of corn and timothy in tubes of sterilized soil, to which were added pure cultures of a number of different organisms, resulted in a marked increase in the numbers of these organisms as compared with unplanted soil. Martin (18) showed that a marked specificity was exhibited by alfalfa and sweet clover in their effects on the relative numbers of different kinds of fungi in the soil. Starkey (28) found an increase in numbers of fungi in the neighbourhood of plant roots over the numbers one foot distant to vary from 6% to 80%. The superficial layers of the roots of corn, mangel beet and bean showed very marked increases even over the soil in close proximity to the main root (30). It seemed to be the quality rather than the quantity of root excretions that had the greater effect (29).

Thom and Humfeld (33) grew corn on a number of different soils and made dilution plate counts of the numbers of bacteria at different distances from the roots and on the plant roots themselves. Determination of pH showed that the roots themselves always tended to center around neutrality, and that as a rule this effect is carried to the adjacent soil as well. The writers concluded that the increased numbers of organisms about the plant roots was due to the formation of this neutral zone, but their conclusions do not seem to be fully borne out by the figures. The greatest increase of all was found in Carrington loam, and yet there the pH of the soil and of the soil adjacent to the roots was 7.0, while that of the fibrous roots themselves which had seven times as many bacteria as the adjacent soil was 7.6. Lyon and Wilson (17) found that during their growth plants liberate quite large quantities of organic matter, and this, forming available nutrient material, seems to be the most satisfactory explanation of the increased activities of microorganisms in the neighbourhood of plant roots.

Experimental Methods and Results

It was considered that it would be of value to determine whether the material liberated from plants susceptible to foot-rots and those resistant differ in the extent to which they influence the growth of foot-rotting

fungi. In order to conduct experiments it was necessary to obtain a larger amount of material than could be obtained from the sloughed off material of plants grown in flasks. Since this sloughed off material consists mainly of plant cells, it seemed reasonable to conclude that similar material could be obtained by crushing up the entire roots of various species of grasses. In addition, it was necessary to cut down the work as much as possible by using only one organism, namely Ophiobolus graminis.

In the first experiment attempts were made to determine not only if there were any effects on the growth of O. graminis by adding extracts of crushed plant roots, but also to determine whether such plant roots, after decomposition by other organisms, would continue to have an influence.

Pots of sterilized soil were seeded with Agropyron repens, A. tenerum, Triticum vulgare and Phleum pratense. These plants were grown for more than three months and the roots were then removed, thoroughly washed, and air dried. As the amounts of root material of wheat and timothy were small, they were supplemented by roots of the same plants grown on unsterilized soil. No diseased roots were observed in any instance and no rhizomes had formed on plants of A. repens. After being thoroughly dried, the roots were ground up to a fine powder, weighed out into half-gram lots and placed in test tubes. In addition, checks were made up

similarly with filter paper, well ground up, to be used with a nutrient solution. To each of these check tubes was added 5 cc. of Richard Duggar's solution made up as follows:

KNO_3	10 gm.
KH_2PO_4	5 gm.
$MgSO_4.7H_2O$	2.5 gm.
Sucrose	50 gm.
Distilled water	1000 cc.

To the tubes of ground roots just sufficient distilled water was added so that the material would soak it up and leave no water lying on the surface. The following amounts were therefore added:

Agropyron repens	5.5 cc.
A. tenerum	5.0 cc.
Triticum vulgare	3.5 cc.
Phleum pratense	3.5 cc.

All the tubes were then placed in the autoclave for one-half hour at 15 pounds pressure. They were then inoculated with two saprophytic organisms, namely Penicillium cyaneo fulvum and a species of Rhizopus obtained from the surface of a wheat kernel.

After twelve days 15 cc. of distilled water were added to each test tube, which was shaken for about one minute and then filtered immediately through filter paper. The liquids from the test tubes in each replicate were bulked, and the 15 different solutions were then sterilized

similarly with filter paper, until dry-out, in an open air
a nutrient solution. To each of these glass dishes was added
5 cc. of Stoddard Dodgen's solution made up as follows:

KNO_3	10 gm.
KH_2PO_4	5 gm.
$MgSO_4.7H_2O$	5.0 gm.
Water	01 gm.
Distilled water	1000 cc.

To the tubes of treated soils just sufficient
distilled water was added so that the material would
it up and leave no water lying on the surface. The following
bacteria were inoculate added:

Azotobacter vinelan	5.0 cc.
B. megaterium	5.0 cc.
Nitrosomonas europaea	5.0 cc.
Nitrobacter agilis	5.0 cc.

All the tubes were then placed in the autoclave
for one-half hour at 15 pounds pressure. They were then
incubated with two sterilized controls, namely
Lactobacillus casei and a species of Nitrosomonas
obtained from the surface of a water normal.

After every days 15 cc. of distilled water was
added to each test tube, which was covered the sheet cot
sterile and then filtered immediately through filter paper.
The liquid from the test tubes in each replicate was
mixed, and the 14 different solutions were then sterilized

by means of Berkefeld filters. Into a number of petri dishes were poured 5 cc. of each extract, as many replicates being made as material would allow, the number varying from as low as 2 in the case of A. tenerum with no organism up to 19 in the case of wheat with P. cyaneo fulvum. In addition 5 cc. of sterile water was added to a number of plates as checks. Then to each plate was added 15 cc. of sodium nitrate agar (34) made up as follows:

Sodium nitrate ($NaNO_3$)	2.0 g.
KH_2PO_4	1.0 g.
KCl	0.5 g.
$MgSO_4.7H_2O$	0.5 g.
$FeSO_4$	0.01 g.
Sucrose	30.0 g.
Distilled water	1000 cc.
Agar	20.0 g.

The dishes were planted with tiny portions of Ophiobolus graminis IV, using as small pieces as could be conveniently obtained from the surface of an agar slant culture. Young cultures of equal age were used for the purpose.

After 9 days growth at 20°C. measurements were taken of the diameter of the colonies. Any plates showing extensive contamination were discarded. The type of colony showed very little variation except in the case of the water check and the Richard Duggar's solution checks, in which cases growth was always much weaker than when root extract

was added. The average measurements of the diameters of the colonies are found in table 5.

Table 5

Stimulation of Growth of *Ophiobolus graminis* on Sodium Nitrate Agar by Root Extracts and Extracts of Partially Decomposed Roots.

Plant roots or nutrient medium used for growth of saprophytic fungi, and for extracting	Average diameter of colonies in centimeters			
	Check. No decomposing organism	Decomposed by *Penicillium*	Decomposed by *Rhizopus*	Average diameter
A. repens	5.6	7.7	5.3	6.2
A. tenerum	7.4	5.5	7.8	6.9
P. pratense	6.1	3.4	6.9	5.5
T. vulgare	5.8	6.4	7.4	6.5
Average	6.2	5.8	6.8	6.3
Check (filter paper and Duggar's solution)	3.6	5.8	3.5	4.3
Water check	2.2			2.2

From these results the following conclusions may be drawn:

1. There was in all cases a stimulation in growth caused by addition of root extracts as compared with checks using sterilized distilled water added directly to the agar in the dishes, and also in the instances where Duggar's solution was added to filter paper and treated in a manner

similar to the roots, with the possible exceptions of the cases noted below in (4).

2. On the average, the Duggar's solution check showed growth of double the diameter of growths in water checks and the fungus on agar which had root extracts added showed 50% increase over the filter paper Duggar's solution extracts.

3. The differences between different root extracts were on the average no greater than the differences between different organisms.

4. There was less growth with extracts of A. tenerum and Phleum pratense with Penicillium than there was with Duggar's solution and Penicillium.

5. With the exceptions noted above in (4), it did not appear that the decomposition products of the roots and the staling products of these two fungi themselves had any appreciable stimulatory or inhibitory effect on the growth of Ophiobolus graminis as compared with extracts which had not been decomposed.

These results suggest that possibly the presence of roots in the soil, even roots of plants not susceptible to take-all, may stimulate the growth of O. graminis in the soil, and that the decomposition products of these roots when decomposed by the two organisms studied, and the staling products of these organisms themselves, will not affect seriously this stimulation. It must be realized that other organisms might give different results.

These results led to a further and more extensive study of the stimulatory effects of root extracts on O. graminis. The following grasses and cereals were grown in sterilized soil for three months: Agropyron repens, A. tenerum, Triticum vulgare, Avena fatua, Bromus inermis, Phleum pratense and Hordeum jubatum. They were then air-dried and ground to powder in a Wylie Mill. To 20 grams of root material of each species was added 300 cc. of distilled water. The flasks were well shaken and then allowed to stand for 18 hours at a temperature of 5°C., which was considered too low for any appreciable fermentation to take place. The extracts were then sterilized by means of Berkefeld filters, and distilled water was sterilized in a similar manner for use as checks. By means of sterile pipettes 5 cc. of extract, or water in the case of the checks, was added to each of a series of petri dishes. Thus each petri dish contained the equivalent of the extract from one-third gram of plant root material.

Two types of agar were used in the study, one being potato dextrose agar giving a dense growth of O. graminis which should show to a marked degree if any inhibitory action were present; the other, soil extract agar, a poor medium for the growth of O. graminis, and one therefore well suited to illustrate a stimulatory effect. Both types of agar were made up according to the formula of Fred and Waksman (9), except that only three quarters of the

normal amount of water was used, thus making due allowance for the extract added separately to the dishes. Edmonton black loam was used in making the soil extract agar.

The potato dextrose agar consisted of:

Agar	30.0 gm.
Potato	200.0 gm.
Dextrose	20.0 gm.
Tap water	750.0 cc.

The soil extract agar had the following composition:

Agar	12.5 gm.
Glucose	1.0 gm.
Dipotassium phosphate (K_2HPO_4)	0.5 gm.
Soil extract (stock)	100.0 cc.
Tap water	650.0 cc.

The stock solution of soil extract was prepared by heating 1000 grams of Edmonton black loam soil with 1000 cc. of tap water in the autoclave for thirty minutes. A small amount of calcium carbonate was added and the whole was filtered through a double paper filter.

It will be seen that both media contained only 750 cc. of water instead of 1 liter. As 15 cc. of agar were added to each dish containing 5 cc. of root extract, the water content of the agar was brought up to normal.

The colonies were measured after seven days growth, and table 6 shows the average diameters of the colonies in centimeters in descending order of magnitude.

Table 6

Effects of Root Extracts on Growth of *Ophiobolus graminis*.

Potato dextrose agar			Soil extract agar		
Number of colonies	Average diameter of colonies in cms. Plant roots	Diameter	Number of colonies	Average diameter of colonies in cms. Plant roots	Diameter
14	*Avena fatua*	3.9	14	*Hordeum jubatum*	4.5
9	*Phleum pratense*	2.6	14	*Agropyron tenerum*	4.1
13	*Triticum vulgare*	2.3	16	*Triticum vulgare*	3.7
21	Water check (No root extract)	2.3	14	*Phleum pratense*	2.6
14	*Agropyron tenerum*	2.2	15	*Avena fatua*	2.5
7	*Agropyron repens*	2.1	19	Water check (No root extract)	2.1
15	*Hordeum jubatum*	2.0	12	*Agropyron repens*	1.9
13	*Bromus inermis*	2.0	14	*Bromus inermis*	1.5

It was noticed that there was a marked difference in the density of mycelium in different series. On soil extract agar the growth was much less dense in all cases than on potato dextrose agar; this was especially true of the water check, which showed so thin a growth that on a basis of actual weight of mycelium produced it would certainly be below all the others.

Table 8

Effects of Root Extracts on Growth of Cercobolus
Crenulatus.

Number of colonies	Potato dextrose agar		Soil extract agar	
	Average diameter of colonies in cms. after three days	Number of colonies	Average diameter of colonies in cms. after three days	Number of colonies
14	Neem Tree	8.9	14	Neem Tree
9	Tulsi Leaves	8.3	14	Tulsi Leaves
12	Citrus Tree	8.3	14	Citrus Tree
21	Water Check (No root extract)	8.9	14	Citrus Tree
14	Lantana Leaves	8.2	12	Lantana Leaves
7	Aloe Vera Leaves	7.4	10	Water Check (No root extract)
12	Tobacco Leaves	8.0	12	Aloe Vera Leaves
12	Guava Leaves	7.0	12	Guava Leaves

Owing to a very considerable increase in growth the colonies were again measured three days later, and in order to obtain as nearly as possible a true comparison of amount of growth, an arbitrary system was adopted in which results could be expressed in terms of "growth index" as well as directly in order of diameter of the colonies. This "growth index" was obtained by placing the colonies in five classes according to density of growth. These classes were valued at densities of 1 to 5, and the growth index of each series is the average growth index of all the colonies in the series; each individual index being the product of the diameter of the colony multiplied by the class value. It is not suggested that such a grading will give a true comparison in all cases; certainly it is not suitable for comparing amounts of growth on the two different types of medium. In other words, this "growth index" is merely an arbitrary scheme used in an attempt to place on a mathematical and tangible basis results which cannot be suitably expressed as a measurement of a single variable such as diameter of the colony. As will be seen in table 7, the results do not differ greatly from those obtained three days previously; where the order has been changed the differences are small except in the case of the water check on soil extract agar, which on a basis of actual weight of mycelium would undoubtedly have shown the least growth in both cases.

Table 7

Average Diameters of Colonies and Growth Index of *Ophiobolus graminis* after Ten Days, Showing Stimulation by Root Extracts.

Root extract	Potato dextrose agar			Root extract	Soil extract agar		
	No.of colonies	Average diameter in cms.	Growth index		No.of colonies	Average diameter in cms.	Growth index
Avena fatua	10	6.2	31.0	*Hordeum jubatum*	14	6.9	20.3
Phleum pratense	7	4.2	20.9	*Agropyron tenerum*	11	6.0	18.9
Hordeum jubatum	11	3.4	16.5	*Triticum vulgare*	15	6.0	18.0
Agropyron tenerum	10	3.2	16.2	*Phleum pratense*	14	4.6	13.7
Triticum vulgare	8	3.0	15.2	*Avena fatua*	9	4.5	13.5
Water	18	3.0	15.0	*Agropyron repens*	10	3.4	10.4
Bromus inermis	8	2.5	12.5	*Bromus inermis*	12	3.1	9.2
Agropyron repens	6	2.4	12.3	Water	19	3.0	4.2

Typical colonies of the various series are shown in Plate VI.

While in detail the results do not entirely agree with those of the previous experiment, the general principle remains the same. The soil extract agar used in this experiment corresponds roughly to the sodium nitrate agar

Table V

Average diameters of colonies and Growth Index of Ophiobolus graminis after Ten Days, showing stimulation by root Extracts.

Root extract	No. of colonies measured	Colony destroyed after Average diameter in cms.	Growth Index	Root extract	No. of colonies measured	Daily growth index after ten days.
Avena fatua	10	6.2	31.0	Avena fatua	12	1.1
Phleum pratense	7	4.8	24.0	Phleum pratense	11	2.0
Dactylis glomerata	11	7.6	38.0	Dactylis glomerata	11	2.0
Lycopersicum esculentum	10	7.8	39.0	Lycopersicum esculentum	12	2.1
Trifolium pratense	8	7.0	35.0	Trifolium pratense	9	1.5
Vetch	10	8.0	40.0	Vetch	12	2.1
Brassica juncea	8	7.0	35.0	Brassica juncea	12	2.1
Agropyron repens	8	8.4	42.0	Quack	12	2.1

Typical colonies of the various hosts are shown in Plate VI.

While in detail the results do not entirely agree with those of the previous experiment, the general principle remains the same. The soil extract used in this experiment contained roughly 5% the organic matter

PLATE VI

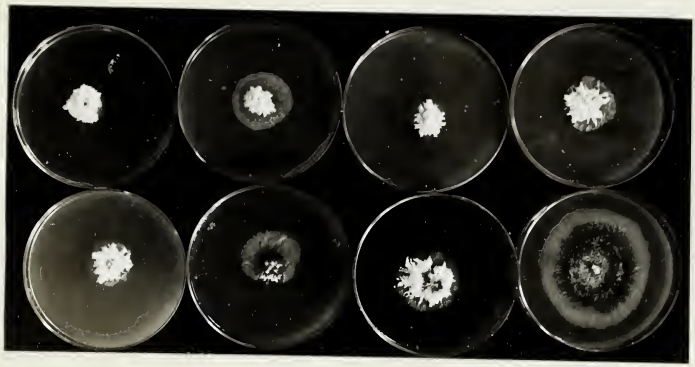

Figure 1

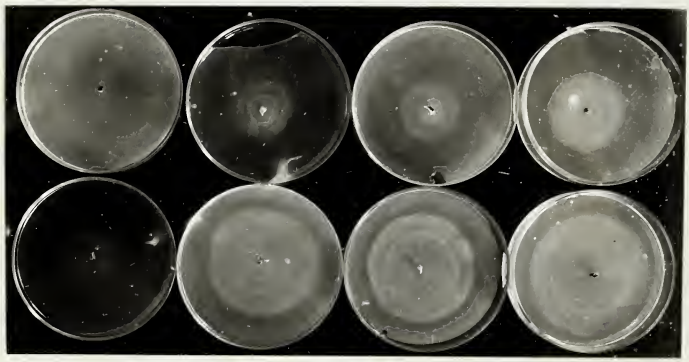

Figure 2

Effect of root extracts of certain grasses on growth of Ophiobolus graminis in pure culture. Figure 1, growth on potato dextrose agar. Left to right, top row; extracts of Bromus inermis, Hordeum jubatum, Agropyron repens, A. tenerum. Bottom row; water (control), wheat, Phleum pratense, Avena fatua. Figure 2, growth on soil extract agar. Left to right, top row; water (control), Bromus inermis, Agropyron repens, Avena fatua. Bottom row; Phleum pratense, wheat, Agropyron tenerum, Hordeum jubatum.

used in the previous one, but contains much less carbohydrates. Comparing the descending list on soil extract agar in this experiment with table 5, column 2, it is seen that in both cases extract from Agropyron tenerum causes greatest stimulation of growth of Ophiobolus graminis, and that from Agropyron repens causes least of the four extracts under consideration. Triticum vulgare and Phleum pratense extracts have interchanged their position in the second experiment, but the difference in the first experiment was small and not significant.

The most striking result was the stimulation of growth of O. graminis by extract of Avena fatua on potato dextrose agar. Avena fatua is believed not to be susceptible to take-all. The main points brought out by the second experiment are:

1. On potato dextrose agar, which is a good nutritive medium and contains ample carbohydrates for growth of O. graminis, the only plant extracts stimulating growth are those from wild oats and timothy, both believed to be highly resistant to O. graminis, and in no case does there appear to be any detrimental effect produced by the extract.

2. On soil extract agar there is considerable stimulation of growth by all extracts as compared with the extremely weak growth on the water check, and the amount of stimulation does not correspond with the susceptibility of the plants to attack by O. graminis.

In all the cases studied the addition of extracts from plant roots to media on which O. graminis was growing resulted in increased growth of this fungus when the nutritional value of the medium was low, and while the different plants gave varying degrees of stimulation, this could not in any way be correlated with the susceptibility of the grass to take-all. This suggests, therefore, that accumulation of dead root material even of non-susceptible grasses may possibly cause an increased growth of O. graminis. It must, however, be remembered that in the soil a great many complicating factors may arise to upset this influence.

It seems also reasonable to conclude that since in no case is any inhibition of growth by non-susceptible species recorded, but rather a stimulation of greater or lesser extent; resistance to attack by O. graminis is not due to the production by the higher plant of a chemical substance which remains toxic after the death of the tissue. If a toxic substance is produced, it evidently loses its toxicity very soon after the death of the plant tissue.

Finally, it might be mentioned that while these results are by no means conclusive, it does not on the surface appear as if there would be any great likelihood of actually causing an inhibition of growth of O. graminis in the soil by growing non-susceptible plants. On the other hand, this does not in any way affect the general principle shown by other experiments, that in order to keep take-all in check, it is necessary to use rotations which at certain

periods in the cycle will cause starving out of the parasite owing to lack of a medium on which it can grow successfully to the exclusion of saprophytic microorganisms.

GENERAL DISCUSSION

A number of grasses of the genus Agropyron are distributed over the province of Alberta, some growing as native grasses and weeds, and others under cultivation. One of the most common of these weeds is A. repens, while A. tenerum is cultivated to a considerable extent, and A. cristatum is likely to become important as a forage crop, especially in the drier parts. Bromus inermis is cultivated extensively particularly in central Alberta. The use of grasses as forage crops is likely to become more widespread as time goes on. At the same time, rotations have been urged for many of the problems of the farmer, such as the maintenance of the fertility of the soil, and the prevention of soil drifting. Another plausible argument which has been advanced in favour of rotations is that they tend to reduce damage from foot- and root-rots.

Many of the rotations suggested involve the use of grasses, such as western rye grass and brome grass. Furthermore, grass mixtures often allow the increase of weed grasses such as Agropyron repens. If, then, such

grasses serve in any way to increase the quantity of foot-rotting organisms in the soil, it is hardly to be expected that farming methods involving their use will help in solving the foot-rot problem.

All the evidence is to the effect that susceptible species tend to aid O. graminis to survive in the soil, to increase the amount of inoculum of this organism for succeeding wheat crops, and to aid greatly its spread in the soil. In the case of H. sativum the effects are somewhat less marked, but there is sufficient evidence to conclude that most species of Agropyron as well as Bromus inermis increase the amount of inoculum of this organism in the soil, and since the fungus seems to be of widespread distribution, it seems probable that such grasses will play a part in the foot-rotting of wheat by this organism also. With regard to F. graminearum, there seems to be a fair amount of evidence that Agropyron species do not appreciably increase the amount of inoculum or rate of spread of this organism. Bromus inermis, on the other hand, may be of importance, at least in increasing the amount of inoculum.

Little work has been done with non-susceptible species, and it is impossible yet to draw any definite conclusions, though there is evidence obtained from extract experiments that even such species may serve to stimulate growth of O. graminis. On the other hand, non-pathogenic

fungi would probably be stimulated to an equal or greater extent. No evidence has yet indicated that resistant or immune species can be expected actually to have a detrimental effect on the foot-rotting fungi.

Survey work conducted in the province has indicated that the damage caused by take-all is probably greater than that caused by other foot-rotting organisms, and for this reason special emphasis should be laid on the take-all problem. It is evident from the results that in order to control take-all, susceptible species should be avoided or used sparingly in rotations. Experimental results and field observations show that old stands of western rye grass have a marked effect upon the accumulation of O. graminis, and for this reason cultivation of this crop should not be encouraged where take-all is a serious problem. It has been seen that A. repens occurs often even in fallow fields where other weeds have been kept under control. Rotations which do not involve eradication of this grass, and summerfallow methods which do not destroy it, cannot be expected to solve the problem, and may even intensify it. From knowledge that has accumulated so far it would seem that awnless brome grass may perhaps be a lesser offender in this respect than western rye grass, but it certainly is not entirely free from blame. There is reason to suppose that timothy does not increase foot-rots of wheat.

The experiments shed light upon the manner in which O. graminis spreads in the soil. This organism spreads little, if at all, in bare soil, but in soil supporting growth of susceptible grasses it spread to a considerable distance. It is true that the greatest spread which occurred was only twelve inches in six weeks, but when it is considered that during this short period it had to establish itself upon healthy young plants in the soil and pass from plant to plant by means of a fine network of roots; that no attempt was made to provide ideal conditions for the growth of the organism in the soil; that previous attempts to demonstrate the spread of the organism had resulted in failure; and that in the unplanted soil the degree of infection of wheat seedlings grown actually in the inoculum only six weeks after it was applied was much lighter than that of plants two and four inches distant in planted soil, there is abundant evidence that susceptible species aid greatly the spread of the organism, if, indeed, they are not absolutely essential.

When we add to all this the fact that a stand of western rye grass may itself become so severely attacked by O. graminis as to be largely destroyed and permit the entrance of numerous noxious weeds, it is seen that there are good reasons to consider seriously the advisability of discouraging the use of this crop for forage purposes in the foot-rot areas. A further study should undoubtedly

be made of the damage caused to stands of this grass with increasing age, as it seems possible that fields left down for only two or three years may not be a great menace.

It is suggested that before any grass be recommended for a forage crop in the foot-rot areas it should first be thoroughly investigated by some such methods as those followed in this investigation to determine its capabilities in regard to the survival, accumulation, and rate of spread of plant pathogenes. Even some of those grasses at present in cultivation should be investigated further. Amongst these is brome grass, which should be more carefully studied under natural conditions in the field. Rotation studies, and the history of infested fields, should yield valuable additional information.

There seems to be little doubt that adoption of rotations, including crops not susceptible to attack by wheat foot-rotting organisms, and the use of really clean summerfallow, will be of great value in reducing the foot-rot problem.

SUMMARY

1. Higher plants are known to have a marked effect on the abundance, activity and type of microorganisms in the soil.

2. Many species of Agropyron and other grasses are known to be susceptible to attack by wheat foot-rotting organisms. It was desired to determine whether these grasses are of significance in the wheat foot-rot problem in Alberta.

3. Organisms chosen for study were Ophiobolus graminis Sacc., Helminthosporium sativum P.K.B., and Fusarium graminearum Schwabe.

4. Agropyron tenerum, A. cristatum, A. repens and Bromus inermis aided the survival of O. graminis in unsterilized soil, and increased the infection of the wheat crop following with this organism on both sterilized and unsterilized soil.

5. All four grasses increased the infection of the succeeding wheat with H. sativum to a marked degree on sterilized soil, but only slightly on unsterilized soil.

6. The only grass studied which increased the infection of the succeeding wheat with F. graminearum was Bromus inermis, which increased infection on unsterilized soil.

7. Neslia paniculata (ball mustard) apparently had no effect on the amount of foot-rot of the wheat following it.

8. The amount of damage of grass roots with foot-rot when grown under varied experimental conditions appears to give some indication of the importance of the grass in the foot-rot problem.

9. *O. graminis* did not appear to spread in unsterilized, bare soil, but spread at varying rates in soil planted to wheat, *Agropyron tenerum* and *A. repens*.

10. The role of grasses in the overwintering of wheat foot-rotting organisms, although not studied experimentally in this investigation, would appear on the surface not to be essentially different from that holding during the summer.

11. Extracts of roots of *Agropyron repens*, *A. tenerum*, *Phleum pratense* and wheat partially decomposed by *Penicillium cyaneo fulvum* and *Rhizopus* sp. caused stimulation of growth of *O. graminis* on sodium nitrate agar. As a rule, the partial decomposition of the roots by fungi tested did not seem to reduce the stimulatory effect.

12. Unheated root extracts of a number of grasses stimulated growth of *O. graminis* on potato dextrose agar and soil extract agar. There seemed to be no correlation between susceptibility of the species and the extent to which it stimulated *O. graminis* in pure culture.

13. It is possible that even resistant species liberate in the soil substances stimulating growth of O. graminis, but probably this effect is masked by other organisms.

14. No evidence was obtained that resistant plants have an inhibitory effect on the cereal foot-rotting fungi in the soil.

15. Rotations should not be expected to aid in the control of foot-rots of wheat if susceptible grasses such as western rye grass (Agropyron tenerum) are included in the rotation.

16. Agropyron repens occurs commonly as a weed in many parts of Alberta, even in summerfallow fields, and without its destruction take-all cannot be controlled.

17. The summerfallow is valueless in controlling foot-rot unless susceptible weeds are destroyed.

18. It is suggested that newly introduced grasses should be thoroughly investigated in relation to the foot-rot problem before being recommended as forage crops.

ACKNOWLEDGEMENTS

The writer wishes to express his thanks to Dr. A. W. Henry for advice given throughout the investigation, and for helpful suggestions and criticisms in preparing this manuscript; also to the National Research Council for financial assistance received.

13. It is possible that even resistant species liberate in the soil substances stimulating growth of *O. graminis*, but probably this effect is masked by other organisms.

14. No evidence was obtained that resistant plants have an inhibitory effect on the usual foot-rotting fungi in the soil.

15. Rotations should not be expected to aid in the control of foot-rots of wheat if susceptible grasses such as western rye grass (*Agropyron tenerum*) are included in the rotation.

16. *Agropyron repens* occurs commonly as a weed in many parts of Alberta, even in summerfallow fields, and without its destruction foot-rot cannot be controlled.

17. The summerfallow is valueless in controlling foot-rot unless susceptible weeds are destroyed.

18. It is suggested that newly introduced grasses should be thoroughly investigated in relation to the foot-rot problem before being recommended as forage crops.

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The writer wishes to express his thanks to Dr. A. W. Henry for advice given throughout the investigation, and for helpful suggestions and criticism in preparing this manuscript; also to the National Research Council for financial assistance received.

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APPENDIX I

Influence of *Agropyron tenerum* on the severity of foot-rot infection in the following wheat crop.

1	2	3	4	5	6	7
Soil	Organism	Weed	Av. height in cms.	Probability	Av. % degree infection	Probability
Unsterilized	*Ophiobolus*	Absent	22.3	9999:1	0	4999:1
Unsterilized	*Ophiobolus*	Present	17.9		11.3	
Sterilized	*Ophiobolus*	Absent	24.8	30:1	0.4	4999:1
Sterilized	*Ophiobolus*	Present	22.1		13.0	
Unsterilized	*Helminthosporium*	Absent	22.0	22.5:1	9.4	22.5:1
Unsterilized	*Helminthosporium*	Present	23.6		14.5	
Sterilized	*Helminthosporium*	Absent	22.4	>9999:1	3.3	>9999:1
Sterilized	*Helminthosporium*	Present	17.6		14.7	
Unsterilized	*Fusarium*	Absent	14.4	100:1	17.5	8:1
Unsterilized	*Fusarium*	Present	11.7		23.0	
Sterilized	*Fusarium*	Absent	12.0	2:1	30.6	4:1
Sterilized	*Fusarium*	Present	12.0		26.1	

APPENDIX II

Influence of *Agropyron cristatum* on the severity of foot-rot infection in the following wheat crop.

1	2	3	4	5	6	7
Soil	Organism	Weed	Av. height in cms.	Probability	Av. % degree infection	Probability
Unsterilized	*Ophiobolus*	Absent	22.3	4:1	0	81:1
Unsterilized	*Ophiobolus*	Present	22.4		1.8	
Sterilized	*Ophiobolus*	Absent	24.8	3:1	0.4	4999:1
Sterilized	*Ophiobolus*	Present	24.2		5.8	
Unsterilized	*Helminthosporium*	Absent	22.0	1:1	9.4	22.5:1
Unsterilized	*Helminthosporium*	Present	22.3		15.3	
Sterilized	*Helminthosporium*	Absent	22.4	216:1	3.3	>9999:1
Sterilized	*Helminthosporium*	Present	19.4		9.8	
Unsterilized	*Fusarium*	Absent	14.4	22:1	17.5	5:1
Unsterilized	*Fusarium*	Present	12.0		22.2	
Sterilized	*Fusarium*	Absent	12.0	37:1	30.6	8:1
Sterilized	*Fusarium*	Present	13.2		20.2	

APPENDIX III

Influence of *Agropyron repens* on the severity of foot-rot infection in the following wheat crop.

1	2	3	4	5	6	7
Soil	Organism	Weed	Av. height in cms.	Probability	Av. % degree infection	Probability
Unsterilized	*Ophiobolus*	Absent	22.3	18:1	0	216:1
Unsterilized	*Ophiobolus*	Present	20.7		5.2	
Sterilized	*Ophiobolus*	Absent	24.8	1:1	0.4	1999:1
Sterilized	*Ophiobolus*	Present	24.7		6.7	
Unsterilized	*Helminthosporium*	Absent	22.0	13.6:1	9.4	49.3:1
Unsterilized	*Helminthosporium*	Present	23.1		15.4	
Sterilized	*Helminthosporium*	Absent	22.4	>9999:1	3.3	344:1
Sterilized	*Helminthosporium*	Present	17.4		9.6	
Unsterilized	*Fusarium*	Absent	14.4	49:1	17.5	2:1
Unsterilized	*Fusarium*	Present	12.5		20.0	
Sterilized	*Fusarium*	Absent	12.0	1:1	30.6	1:1
Sterilized	*Fusarium*	Present	11.9		32.5	

APPENDIX IV

Influence of *Bromus inermis* on the severity of foot-rot infection in the following wheat crop.

1	2	3	4	5	6	7
Soil	Organism	*B. inermis*	Av. height in cms.	Probability	Av. % degree infection	Probability
Unsterilized	*Ophiobolus*	Absent	22.0	81:1	0	4999:1
Unsterilized	*Ophiobolus*	Present	20.9		9.9	
Sterilized	*Ophiobolus*	Absent	24.8	3:1	0.4	1999:1
Sterilized	*Ophiobolus*	Present	24.2		15.9	
Unsterilized	*Helminthosporium*	Absent	22.2	1110:1	5.6	1999:1
Unsterilized	*Helminthosporium*	Present	17.2		19.1	
Sterilized	*Helminthosporium*	Absent	23.3	10.9:1	31.8	999:1
Sterilized	*Helminthosporium*	Present	22.1		51.0	
Unsterilized	*Fusarium*	Absent	22.7	>9999:1	3.6	>9999:1
Unsterilized	*Fusarium*	Present	14.3		27.1	
Sterilized	*Fusarium*	Absent	19.2	4.11:1	44.9	1:1
Sterilized	*Fusarium*	Present	18.3		41.1	

APPENDIX V

Influence of *Neslia paniculata* on the severity of foot-rot infection in the following wheat crop.

1	2	3	4	5	6	7
Soil	Organism	Weed	Av. height in cms.	Probability	Av. % degree infection	Probability
Unsterilized	*Ophiobolus*	Absent	20.2	1:1	0	--
Unsterilized	*Ophiobolus*	Present	20.0		0	
Sterilized	*Ophiobolus*	Absent	24.8	30:1	0	--
Sterilized	*Ophiobolus*	Present	23.4		0	
Unsterilized	*Helminthosporium*	Absent	18.5	1:1	5.0	1.59:1
Unsterilized	*Helminthosporium*	Present	18.2		4.9	
Sterilized	*Helminthosporium*	Absent	21.5	163:1	30.7	1.59:1
Sterilized	*Helminthosporium*	Present	18.6		30.3	
Unsterilized	*Fusarium*	Absent	20.3	8.34:1	7.2	v.small
Unsterilized	*Fusarium*	Present	19.5		7.8	
Sterilized	*Fusarium*	Absent	18.8	1:1	31.4	6.67:1
Sterilized	*Fusarium*	Present	19.7		21.4	

APPENDIX I

Influence of Depth Periodicity on the Quantity of Food-rat Interaction in the following Water Body.

APPENDIX VI

Results of experiments on the spread of *O. graminis* in flats.

Soil treatment	Distance in inches from inoculum	First replicate			Second replicate			Third replicate		
		Height	% degree infection	Isolations	Height	% degree infection	Isolations	Height	% degree infection	Isolations
Soil left bare. Cornmeal soil, with no organism, placed at one end of box. Included to check against natural occurrence of *O. graminis* in soil.	0	26.9	0	-	26.6	0	-	23.7	0	-
	2	25.2	0	-	28.4	0	-	25.9	0	-
	4	22.1	0	-	27.1	0	-	21.9	0	-
	6	25.4	0	-	21.4	0	-	21.1	0	-
	8	25.9	0	-	23.4	0	-	21.1	0	-
	10	29.0	0	-	22.4	0	-	21.8	0	-
	12	23.5	0	-	20.6	0	-	21.6	0	-
	14	23.3	0	-	23.8	0	-	21.6	0	-
Soil left bare. *O. graminis* placed at one end of box.	0	26.1	14.5	+	20.4	15.0	+	25.9	13.3	+
	2	24.4	0	-	24.0	4.0	+	23.6	0	-
	4	25.6	0	-	24.1	0	-	21.9	0	-
	6	25.3	0	-	22.4	0	-	23.2	0	-
	8	26.0	0	-	22.8	0	-	27.4	0	-
	10	20.4	0	-	24.1	0	-	24.5	0	-
	12	22.5	0	-	21.2	0	-	22.6	0	-
	14	25.8	0	-	22.5	0	-	22.6	0	-

Number of telephones per 100 population in states.

APPENDIX VI (Continued)

Soil treatment	Distance in inches from inoculum	First replicate			Second replicate			Third replicate		
		Height	% degree infection	Isolations	Height	% degree infection	Isolations	Height	% degree infection	Isolations
Soil seeded to wheat. *O. graminis* placed at one end of box.	0	29.2	26.7	+	26.7	10.0	+	30.9	4.0	+
	2	25.4	32.0	+	22.5	42.5	+	23.9	16.0	+
	4	18.2	38.2	+	21.4	24.0	+	20.5	26.2	+
	6	20.7	16.9	+	20.1	10.9	+	17.0	18.2	+
	8	22.0	11.4	-	21.6	8.3	+	22.4	3.1	+
	10	22.3	1.7	-	20.6	0	-	20.5	0	-
	12	20.7	0	-	19.9	0	-	22.9	0	-
	14	22.9	0	-	22.8	0	-	18.9	0	-
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of box.	0	27.2	5.4	+	24.9	14.7	+	25.0	7.3	+
	2	22.6	32.3	+	19.5	27.7	+	22.9	13.3	+
	4	22.2	10.0	+	20.6	13.3	+	20.9	9.1	+
	6	20.0	0	-	17.0	25.5	+	19.4	1.8	+
	8	19.3	0	-	17.4	17.8	+	21.2	2.8	+
	10	16.9	0	-	17.1	1.8	+	21.0	0	-
	12	18.4	0	-	20.6	1.5	+	17.1	0	-
	14	19.4	0	-	18.9	0	-	18.2	0	-
Soil seeded to *A. repens*. *O. graminis* placed at one end of box.	0	24.8	15.3	+	27.7	6.2	+	26.4	4.4	+
	2	29.6	26.7	+	26.5	22.5	+	23.5	2.2	+
	4	21.3	18.7	+	20.8	11.4	+	21.5	6.7	+
	6	21.6	0	-	20.1	3.3	-	20.5	0	-
	8	19.7	0	-	20.4	0	-	21.1	0	-
	10	21.7	0	-	20.6	0	-	20.5	0	-
	12	20.8	0	-	19.4	0	-	21.5	0	-
	14	16.5	0	-	18.5	0	-	19.1	0	-

APPENDIX V2 (continued)

Soil treatment	Distance in inches from inoculum	First replicate Height %	First replicate Tap-root lesions	Second replicate Height %	Second replicate Tap-root lesions	Third replicate [illegible] %	Third replicate Tap-root lesions
Soil soaked to	0	22.2	7C.7	-	5C.7	10.0	+	46.0	4.0	+
wheat. D.	2	21.4	3C.0	+	2C.5	48.3	+	24.4	12.0	+
<u>Pythium</u> placed	4	18.1	3C.2	+	21.4	2C.0	+	3C.4	12.2	+
at one end of	6	3C.7	12.0	+	3C.1	12.0	+	17.0	12.8	+
box.	8	2C.3	11.4	-	21.0	0.2	-	3C.4	8.1	+
	10	2C.2	1.7	-	3C.4	0	-	3C.0	2	-
	12	2C.7	0	-	1C.8	0	-	4C.9	0	-
	14	2C.4	0	-	3C.3	0	-	12.4	0	-
Soil soaked to	0	27.2	2.2	-	3C.1	12.7	-	32.0	7.0	-
4. <u>Pythium</u>.	2	2C.6	32.2	-	1C.0	27.7	-	4C.2	12.2	-
D. <u>Pythium</u>	4	2C.2	12.0	+	3C.1	12.2	+	22.0	7.1	+
placed at one	6	2C.0	0	-	17.0	25.0	-	1C.4	1.2	+
end of box.	8	12.2	0	-	17.4	17.2	-	21.2	0.2	+
	10	1C.2	0	-	17.1	1.2	-	23.0	0	-
	12	12.4	0	-	2C.2	1.2	-	27.1	0	-
	14	12.4	0	-	12.2	0	-	1C.2	0	-
Soil soaked to	0	14.0	12.2	-	3C.1	0.2	-	2C.4	1.4	-
4. <u>Fusarium</u>.	2	2C.2	22.2	+	3C.2	22.2	+	3C.2	2.2	+
D. <u>Pythium</u>	4	21.2	1C.2	+	2C.2	11.4	+	21.2	0.2	+
placed at one	6	21.0	0	-	2C.1	2.2	-	2C.2	0	-
end of box.	8	12.2	0	-	2C.4	0	-	2C.2	0	-
	10	11.2	0	-	2C.2	0	-	2C.2	0	-
	12	2C.0	0	-	1.4	0	-	21.2	0	-
	14	12.2	0	-	2C.2	0	-	2C.2	0	-

APPENDIX VII

Results of experiments on the spread of F. graminearum in flats.

Soil treatment	Distance in inches from inoculum	First replicate			Second replicate			Third replicate		
		Height	% degree infection	Isolations	Height	% degree infection	Isolations	Height	% degree infection	Isolations
Soil left bare. Cornmeal soil with no organism placed at one end of the box. Included to check against natural occurrence of F. graminearum in the soil.	0	27.4	0	- -	33.0	0	- -	30.8	2.7	- -
	2	23.9	0	- -	28.4	8.0	- H	28.0	5.5	- H
	4	22.9	0	- -	30.7	0	- -	30.5	3.6	- -
	6	18.5	0	- -	27.7	0	- -	29.9	2.9	- -
	8	23.0	0	- -	23.3	8.3	- H	27.5	5.7	- H
	10	24.6	0	- -	24.5	6.0	- H	25.0	8.9	- -
	12	22.7	1.4	- -	29.1	1.7	- -	29.3	4.4	- H
	14	24.4	0	- -	29.4	8.0	- H	26.9	2.2	- -
	16	23.6	0	- -	29.1	1.5	- -	30.1	2.5	- -
	18	24.4	0	- -	29.3	0	- -	27.0	10.0	- H
Soil left bare. F. graminearum placed at one end of the box.	0	24.6	5.0	+ -	26.3	20.0	+ H	34.1	13.3	+ -
	2	24.1	0	- -	29.9	9.3	- H	33.1	1.8	- -
	4	25.6	2.4	- H	27.3	0	- -	31.8	1.5	- -
	6	27.0	1.3	- -	26.7	5.7	- -	29.2	0	- -
	8	26.3	1.2	+ -	28.3	3.1	- H	27.1	0	- -
	10	26.0	8.3	- -	27.5	4.0	- H	29.0	2.2	- H
	12	24.2	0	- -	28.6	3.3	- -	27.0	0	- -
	14	25.8	3.6	+ -	27.3	8.6	- H	28.3	6.2	- H
	16	22.1	4.7	+ -	28.2	5.5	- H	27.4	0	- -
	18	24.8	0	- -	28.6	3.3	- H	27.9	0	- -

Results of tests on five steels of a Bessemer or Lister

APPENDIX VII (Continued)

Soil treatment	Distance in inches from inoculum	First replicate			Second replicate			Third replicate		
		Height	% degree infection	Isolations	Height	% degree infection	Isolations	Height	% degree infection	Isolations
Soil seeded to wheat. *F. graminearum* placed at one end of the box.	0	23.5	29.1	+ -	27.9	37.5	+ H	32.7	10.0	+ H
	2	22.3	8.3	- H	30.3	29.3	- H	29.4	10.0	+ H
	4	21.4	14.0	- H	25.8	40.0	- H	27.9	8.9	- H
	6	19.3	9.1	- H	22.9	28.0	- H	26.1	12.3	- H
	8	21.3	10.0	- H	21.4	30.7	- H	23.8	14.3	- H
	10	20.7	8.6	- H	19.4	50.0	- H	22.5	9.2	- H
	12	18.8	7.5	- H	18.8	43.6	- H	19.4	12.0	- H
	14	17.6	11.7	- H	23.0	16.5	+ H	25.5	7.1	- H
	16	21.3	3.3	- -	20.6	35.7	- H	24.3	9.3	- H
	18	20.3	9.2	- -	20.2	36.4	- H	23.4	6.7	- H
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of the box.	0	22.3	16.0	+ H	24.5	49.1	+ H	32.3	18.3	- H
	2	24.4	20.0	+ H	24.9	35.6	+ H	29.5	12.3	- H
	4	23.7	18.7	+ H	24.4	40.0	+ H	28.4	12.9	- H
	6	20.2	25.0	- H	23.8	38.3	- H	26.3	15.0	- H
	8	23.2	10.0	- H	22.6	23.1	- H	22.0	17.3	- H
	10	22.2	0	- -	22.7	18.3	+ H	25.7	14.4	- H
	12	23.7	5.7	- H	24.2	11.4	+ H	24.5	16.0	- H
	14	22.8	7.3	- H	24.0	14.5	- H	25.1	10.9	- H
	16	21.9	1.8	- -	23.8	28.3	- H	21.7	20.0	- H
	18	21.7	12.0	- H	23.6	27.7	- H	23.0	12.0	- H
Soil seeded to *A. repens*. *F. graminearum* placed at one end of the box.	0	21.8	38.0	+ H	31.3	13.3	+ H	27.2	28.0	+ H
	2	22.5	15.6	+ H	25.0	36.9	- H	34.4	26.0	- H
	4	21.6	15.0	- H	27.7	21.4	- H	27.7	35.6	- H
	6	23.3	14.5	- H	25.1	48.3	- H	26.0	28.6	+ H
	8	17.0	40.0	- -	24.6	38.3	- H	25.3	22.9	- H
	10	22.5	21.4	- H	24.3	41.5	- H	27.7	15.0	- H
	12	19.9	32.0	- H	24.9	43.1	- H	27.0	18.0	- H
	14	22.9	11.1	- -	22.5	36.0	- -	25.1	18.3	- H
	16	21.6	4.0	- H	24.8	36.7	- H	25.9	13.3	- H
	18	21.8	6.2	+ H	20.8	31.1	- H	25.4	18.5	- H